

Biostimulation of Iron Reduction and Uranium Immobilization: Microbial and Mineralogical Controls

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Abstract

The overall objective of our project is to understand the microbial and geochemical mechanisms controlling the reduction and immobilization of U(VI) during biostimulation in subsurface sediments of the Field Research Center (FRC) which are cocontaminated with uranium and nitrate. The focus will be on activity of microbial populations (metal- and nitrate-reducing bacteria) and iron minerals which are likely to make strong contributions to the fate of uranium during *in situ* bioremediation. The project will: 1) quantify the relationships between active members of the microbial communities, iron mineralogy, and nitrogen transformations in the field and in laboratory incubations under a variety of biostimulation conditions, 2) purify and physiologically characterize new model metal-reducing bacteria isolated from moderately acidophilic FRC subsurface sediments, and 3) elucidate the biotic and abiotic mechanisms by which FRC aluminosilicate clay minerals are reduced and dissolved under environmental conditions resembling those during biostimulation. Active microbial communities will be assessed using quantitative molecular techniques along with geochemical measurements to determine the different terminal-electron-accepting pathways. Iron minerals will be characterized using a suite of physical, spectroscopic, and wet chemical methods. Monitoring the activity and composition of the denitrifier community in parallel with denitrification intermediates during nitrate removal will provide a better understanding of the indirect effects of nitrate reduction on uranium speciation. Through quantification of the activity of specific microbial populations and an in-depth characterization of Fe minerals likely to catalyze U sorption/precipitation, we will provide important inputs for reaction-based biogeochemical models which will provide the basis for development of *in situ* U bioremediation strategies.

In collaboration with Jack Istok and Lee Krumholz, we have begun to study the change in microbial community composition of FRC sediments during *in situ* biostimulation in single well push-pull tests. Microbial communities were stimulated in the acidic subsurface via pH neutralization and addition of electron donor to wells. Examination of sediment chemistry in cores sampled immediately adjacent to treated wells revealed that sediment pH increased substantially (by 1-2 pH units), while nitrate was largely depleted. Following the *in situ* biostimulation, previously cultured metal-reducing *delta-Proteobacteria* 16S rRNA gene sequences substantially increased from 5% to nearly 40% of clone libraries. Quantitative PCR revealed that *Geobacter*-type 16S rRNA gene sequences increased in biostimulated sediments by one to two orders of magnitude at two of the four sites tested, thereby corroborating information obtained from clone libraries, and indicating that members of the *delta-Proteobacteria* (including *Anaeromyxobacter dehalogenans*-related and *Geobacter*-related organisms) are important metal-reducing bacteria in FRC

Results - In situ Biostimulation of Acidic FRC Subsurface Sediments

Cultivation-independent, Microbial Community Analysis

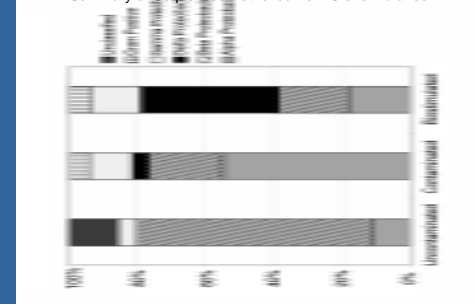


16S rRNA gene sequences retrieved from biostimulated FRC sediment.

FRC Contaminants and Potential Bioremediating Organisms

FRC Contaminant	Physiological potential	Potential bioremediating organisms	Clone library	
			% Before	% After
Uranium	Reduction and immobilization by Fe(II)	<i>Geobacter</i> sp. (5)	4.5%	57.8%
		<i>Anaeromyxobacter dehalogenans</i> (7)		
Nitrate	Reduction and immobilization by Fe(II)	<i>Desulfobacterium autotrophicum</i> (12)	5.7%	18.9%
		<i>Geobacter</i> sp. (10)		
Methane	Reduction	<i>Geobacter</i> sp. (10)	33.8%	27.1%
		<i>Anaeromyxobacter dehalogenans</i> (10)		
Unreduced hydrocarbons	Decomposition	<i>Methylobacterium chloromethanum</i> (23)	42.5%	34.4%
		<i>Anaeromyxobacter dehalogenans</i> (3)		
Polychlorinated biphenyls	Decomposition	<i>Geobacter</i> sp. (10)	14.8%	2.3%
		<i>Geobacter</i> sp. (10)		
Fuel hydrocarbons	Biodegradation	<i>Geobacter</i> sp. (10)	5.7%	14.8%
		<i>Geobacter</i> sp. (10)		

Summary of Sequences Retrieved from Clone Libraries



Conclusions

Iron Mineralogy

- Iron in the FRC subsurface is distributed 64.5 % in iron oxide and 35.5 % in silicate phases (Fig. 1)
- Silicate phases were partially reduced and iron oxides were dissolved during biostimulation (Fig. 2)
- Reliable Mossbauer results for iron phase distribution can be obtained only at 4 K

Microbial Community Analysis

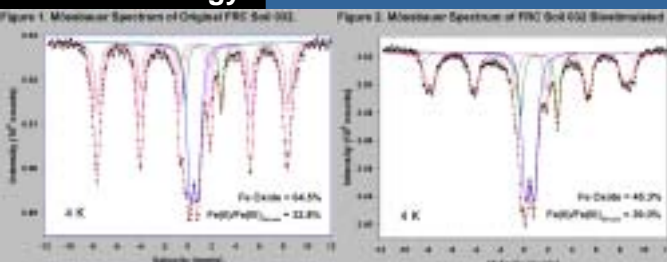
- 16S rRNA gene sequences affiliated with the *delta-Proteobacteria* increased from 5 % to 40 % of clone libraries during biostimulation
- Quantitative PCR revealed that *Geobacter*-type sequences increased by one to two orders of magnitude after biostimulation
- Many of the metal-reducers detected were closely affiliated with cultured organisms (*Geobacter*, *Anaeromyxobacter*, *Desulfobacterium*) capable of coupling the reduction of nitrate, iron, or halogenated compounds to growth

Acknowledgements

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Iron Mineralogy



Mössbauer spectra were obtained using a Webb Research, Inc. spectrometer equipped with a Janis Model SHI-850-5 Closed Cycle Cryostat, operating at a sample temperature of 4 K.



Bioreactor	Organism	Copies 16S gene/g soil (95% Confidence limits)	
		Before biostimulation	After biostimulation
A	<i>Geobacter</i>	5.38×10^3 (4.23 $\times 10^3$; 2.63 $\times 10^3$)	2.63×10^4 (2.19 $\times 10^4$; 9.50 $\times 10^3$)
	<i>Geobacter</i>	5.38×10^3 (4.23 $\times 10^3$; 2.63 $\times 10^3$)	1.50×10^4 (1.06 $\times 10^4$; 7.30 $\times 10^3$)
	<i>Geobacter</i>	2.25×10^3 (1.80 $\times 10^3$; 8.85 $\times 10^2$)	1.50×10^4 (1.06 $\times 10^4$; 7.30 $\times 10^3$)
	<i>Geobacter</i>	5.50×10^3 (4.25 $\times 10^3$; 1.30 $\times 10^3$)	2.80×10^4 (2.38 $\times 10^4$; 1.50 $\times 10^4$)
B	<i>Anaeromyxobacter</i>	2.63×10^3 (2.10 $\times 10^3$; 1.03 $\times 10^3$)	1.10×10^4 (9.75 $\times 10^3$; 5.91 $\times 10^3$)
	<i>Anaeromyxobacter</i>	2.63×10^3 (2.10 $\times 10^3$; 1.03 $\times 10^3$)	1.10×10^4 (9.75 $\times 10^3$; 5.91 $\times 10^3$)
	<i>Anaeromyxobacter</i>	3.45×10^3 (2.25 $\times 10^3$; 1.48 $\times 10^3$)	5.30×10^3 (4.50 $\times 10^3$; 1.79 $\times 10^3$)
	<i>Anaeromyxobacter</i>	4.25×10^3 (3.18 $\times 10^3$; 1.68 $\times 10^3$)	1.10×10^4 (9.75 $\times 10^3$; 5.91 $\times 10^3$)
C	<i>Paenibacillus/Brevibacillus</i>	1.10×10^3 (9.75 $\times 10^2$; 5.91 $\times 10^2$)	1.43×10^3 (9.75 $\times 10^2$; 6.07 $\times 10^2$)
	<i>Paenibacillus/Brevibacillus</i>	1.10×10^3 (9.75 $\times 10^2$; 5.91 $\times 10^2$)	1.00×10^3
	<i>Paenibacillus/Brevibacillus</i>	1.10×10^3	6.90×10^2 (5.85 $\times 10^2$; 3.55 $\times 10^2$)
	<i>Paenibacillus/Brevibacillus</i>	2.30×10^3 (1.85 $\times 10^3$; 7.85 $\times 10^2$)	1.80×10^3 (1.51 $\times 10^3$; 7.30 $\times 10^2$)

Quantitative PCR before and after biostimulation of contaminated sediment with glucose: FRC2 to ready FRC3 (bioreactor set A), FRC3 to ready FRC4 (bioreactor set B), FRC3 to ready FRC5 (bioreactor set C), and ethanol: FRC4 to ready FRC9 (bioreactor set D).